

HUMAN CYTOSKELETAL PROTEINS**TECHNICAL FIELD**

This invention relates to nucleic acid and amino acid sequences of human cytoskeletal proteins and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative, immunological, vesicle trafficking, reproductive, smooth muscle, developmental, and nervous disorders.

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BACKGROUND OF THE INVENTION

The physical-biochemical processes of cell motility, organelle movement, chromosome movement, cytokinesis, and the generation of cell shape are all dependent on a complex of protein fibers found in the cytoplasm. This protein complex is termed the cytoskeleton. The cytoskeleton of eukaryotic cells has three major filamentous systems. These systems are the actin filaments, intermediate filaments, and microtubules. Each of these filamentous systems is assembled from different proteins, including actin, myosin, tubulins, and intermediate filament proteins. Different cell types and tissues express specific isoforms of the proteins which comprise these filaments. In some cases distinct isoforms and mRNA splice variants are associated with cell-type specific functions. (Lees-Miller, J.P. and Helfman, D.M. (1991) BioEssays 13:429-437.)

The actin filamentous system largely regulates cell motility, in particular generation of muscle tissue contraction and relaxation. The actin filamentous system comprises the thick filament and the thin filament. The thick filament is composed of myosin and the thin filament contains actin and a protein complex of troponin and tropomyosin. Activation of myosin binding to actin is initiated by Ca^{2+} -dependent phosphorylation of myosin light chains by Ca^{2+} -dependent protein kinases. This mechanism is termed the primary Ca^{2+} -dependent mechanism. The interaction between actin and myosin which drives muscle contraction is regulated by binding of Ca^{2+} ions to the troponin-tropomyosin complex and is termed the secondary Ca^{2+} -dependent mechanism. The bound troponin-tropomyosin complex inhibits the interaction between actin and myosin at low cellular concentrations of Ca^{2+} ($< 1 \mu\text{M}$). Following a nerve generated signal, Ca^{2+} is released from the sarcoplasmic reticulum (SR). At high levels ($> 1 \mu\text{M}$) Ca^{2+} binds to four sites on troponin and affects the specific molecular interactions between tropomyosin and the actin filament. This reveals the myosin-binding sites on actin, allowing ATP-generated movement of the myosin along the actin filament (contraction). An SR membrane, Ca^{2+} -activated ATPase pumps Ca^{2+} back into the SR thereby returning cytoplasmic Ca^{2+} ion levels to $< 1 \mu\text{M}$. Depletion of cytosolic Ca^{2+} enables the actin-troponin-tropomyosin complex to reform and myosin-actin interactions cease (relaxation). (Reedy, M.K. et al. (1994) Curr. Biol. 4:624-626.)

Different isoforms of tropomyosin have been identified in muscle and non-muscle tissue. The α gene isoform splice variants are in striated and smooth muscle, brain, and fibroblasts; the β chain isoform splice variants are in skeletal muscle and smooth muscle fibroblasts; the hTMnm isoform splice variants are in skeletal muscle and fibroblasts; and the TM-4 isoform splice variant is in rat platelets. (Lees-

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Miller, supra; Pittenger, M.F., et al. (1994) Curr. Biol. 6:96-104.)

Secondary Ca^{2+} -dependent mechanisms can modulate the contractile state of the cell. These secondary mechanisms include, but are not limited to, interactions between i) actin, tropomyosin and calponin; ii) actin, myosin, tropomyosin, and caldesmon; iii) actin, tropomyosin, and titin; iv) actin, tropomyosin, and tropomodulin; and v) protein kinase C-dependent chemical modification of actin filament complexes. (Walsh, M.P. (1991) Biochem. Cell. Biol. 69:771-800; Fowler, V.M. (1997) Soc. Gen. Physiol. Ser. 52:79-89.)

Neuronal development and maturation are accompanied by dynamic spatial sorting of tropomyosin isoforms into different cellular compartments. (Gunning, P. et al. (1997) Anat. Embryol. (Berl.) 195:311-315.) Analysis of the developmental changes in the protein compositions of the brain has identified novel developmentally regulated actin-binding proteins termed debrins. Debrin and tropomyosin competitively bind to actin filaments. The exclusion of tropomyosin from actin filaments by debrin results in the appearance of thick, curving bundles of actin filaments and the formation of cell processes in cultured cells. (Shirao, T. (1995) J. Biochem. (Tokyo) 117:231-236.)

Myocardial performance is impaired in chronically diabetic rats. Malhotra and Sanghi have suggested (1997, Cardiovasc. Res. 34:34-40) that diabetes-associated cardiovascular diseases may involve proteins of the actin filamentous system, in particular myosin and troponin. Phosphorylation of troponin has been associated with altered calcium force in isolated muscle preparations. This may be due to changes in the troponin-tropomyosin-actin complex to prevent or reduce interaction(s) with myosin. It was suggested that phosphorylation of troponin could contribute to depressed myocardial contractility in experimental diabetes. (Malhotra and Sanghi, supra.) Mutations in troponin and tropomyosin are associated with familial hypertrophic cardiomyopathy. (Palmiter, K.A. and Solaro, R.J. (1997) Basic. Res. Cardiol. 92 (suppl.1):63-74.)

The term receptor describes proteins that specifically recognize other molecules. The bulk of receptors are cell surface proteins which bind extracellular ligands and lead to cellular responses including growth, differentiation, endocytosis, and immune response. Cell surface receptors are typically integral membrane proteins of the plasma membrane. These receptors recognize compounds, e.g., catecholamine and peptide hormones, growth and differentiation factors, cytokines, small peptide factors, neurotransmitters, and circulatory system-borne signaling molecules. Cell surface receptors on immune system cells recognize antigens, antibodies, and major histocompatibility complex (MHC)-bound peptide. Other cell surface receptors bind ligands to be internalized by the cell. (Lodish, H. et al. (1995) Molecular Cell Biology, Scientific American Books, New York, NY, p. 723; Mikhailenko, I. et al. (1997) J. Biol. Chem. 272:6784-6791.)

The discovery that the transforming oncogene, *trk*, is a chimeric protein between tropomyosin and the membrane domain and intracellular domain of nerve growth factor receptor has linked mutation of tropomyosin(s) and abnormal expression of tropomyosin(s) to childhood malignancy neuroblastoma.

(Pahlman, S. and Hoehner, J.C. (1996) Mol. Med. Today 2:432-438.) In addition, experimental chimeric proteins containing receptor extracellular and transmembrane domains linked to the integrin β cytoplasmic domain can be induced by exogenous factors to cause cytoskeletal reorganization. (Smilenov, L. et al. (1994) Mol. Biol. Cell 5:1215-1223.) These results show that naturally-occurring and
 5 synthetic chimeric proteins which combine extracellular, transmembrane, and cytosolic elements of otherwise distinct individual proteins have additional and synergistic roles in tissue biology.

Cell motility is governed by the interaction between cytoskeletal and other cellular proteins. Cytoskeletal proteins which are involved in the generation of motive force within the cell are termed contractile proteins. The energy for this force is generated by ATP.

10 Two predominant contractile proteins in all animal cells are actin and myosin. Actin is present in both soluble and polymerized forms. For example, filamentous (polymerized) actin interacts with myosin to contract or relax muscle tissues, to transport cell organelles through the intracellular medium, to cause cell movement, and to separate daughter nuclei during cytokinesis.

Multiprotein complexes associate with actin and myosin in vivo. Actin polymerization can be
 15 initiated, prevented, or reversed by post-translational protein modification and changes in the constituent proteins of the multiprotein complexes. Examples of multiprotein complex constituent proteins include trychohyalin, p16-Arc, and actinin. Trichohyalin is a cross-linking protein that modulates actin polymerization and functions in intermediate filament- nuclear matrix anchoring (Lee, S.C. (1993) J. Biol. Chem. 268:12164-12176). p16-Arc is a subunit of the human Arp2/3 multiprotein complex. The
 20 Arp2/3 complex is localized to the actin-rich lamellipodial protrusions of cells where it is proposed to promote actin assembly and cellular locomotion (Welch, M.D. et al. (1997) J. Cell Biol. 138:375-384). Actinin functions in the linkage of actin to the cell membrane (Honda, K. et al. (1998) J. Cell Biol. 140:1383-1393).

Cytoskeletal proteins are involved in the regulation of muscle contraction. Vertebrate smooth
 25 muscle contraction is dependent upon levels of cAMP and intracellular calcium ions (Ca^{2+}). The sarcoplasmic reticulum (SR) serves as an intracellular store of Ca^{2+} . Following hormonal stimulation of the second messenger molecule, inositoltrisphosphate, Ca^{2+} is briefly released from the SR into the surrounding cytoplasm. Ca^{2+} binds to calmodulin (CaM), which activates CaM-dependent myosin light chain protein kinase (MLCK), which then phosphorylates MLC. In relaxed skeletal muscle, myosin is
 30 prevented from interacting with actin by binding to tropomyosin. An increase in Ca^{2+} causes a conformational change in tropomyosin-actin binding that leads to the release of actin. This allows actin to interact with phosphorylated MLC forming actinomyosin and initiating the contraction process. Muscle relaxation is brought about by active transport of Ca^{2+} into the SR by a calcium ATPase pump and activation of MLCK by a cAMP-dependent protein kinase (PKA). Interactions between MLCK and PKA
 35 may be modulated by other proteins. In particular, telokin, a kinase-related protein encoded by the 3' region of the vertebrate smooth muscle MLCK gene, inhibits MLCK-dependent phosphorylation of MLC

by modulating both the oligomeric state of MLCK and MLCK's interaction with dephosphorylated myosin filaments (Niezanski, K. and Sobieszek, A. (1997) *Biochem. J.* 322:65-71). Caldesmon is a protein involved in smooth muscle contraction that performs a role similar to that of tropomyosin in skeletal muscle. Caldesmon forms a complex with tropomyosin and actin that prevents binding of myosin to actin. Phosphorylation of caldesmon by casein kinase II releases its binding to tropomyosin and actin permitting the cross-linking of myosin to actin and the initiation of smooth muscle contraction (Sutherland, C. et al. (1994) *J. Muscle Res. Cell. Motil.* 15:440-456). Elevation of intracellular cGMP and activation of protein kinase G (PKG) produces relaxation of smooth muscle (Li, H. et al. (1996) *J. Vasc. Res.* 33:99-110).

Cytoskeletal filament proteins which generate cellular movement are components of flagella and cilia. Flagella and cilia are the hair-like structures which protrude from many cells and are composed of proteinaceous cylinders known as axonemes. The major mass of the axoneme consists of tubulins which polymerize to form microtubules. Nine microtubular doublets typically surround, and are linked to, a central pair of microtubules. Intermediate filament proteins, such as tektins, interact with microtubules to regulate movement. Tektins are predicted to form extended α -helical rods capable of forming coiled-coil structures which are interrupted by short non-helical linkers (Norrander, J.M. et al. (1996) *J. Mol. Biol.* 257:385-397). Microtubule-associated proteins (MAPs) regulate cell division and cell motility by modulation of microtubule formation.

Cytoskeletal proteins are implicated in several diseases. Pathologies such as muscular dystrophy, nephrotic syndrome, and dilated cardiomyopathy have been associated with differential expression of alpha-actinin-3 (Vainzof, M. et al. (1997) *Neuropediatrics* 28:223-228; Smoyer, W.E. and Mundel, P. (1998) *J. Mol. Med.* 76:172-183; and Sussman, M.A. et al. (1998) *J. Clin. Invest.* 101:51-61). Alpha-actinin and several MAPs are present in Hirano bodies, which are observed more frequently in the elderly and in patients with neurodegenerative diseases such as Alzheimer's disease (Maciver, S.K. and Harrington, C.R. (1995) *Neuroreport.* 6:1985-1988). Actinin-4, a novel actin-bundling protein, appears to be associated with the cell motility of metastatic cancer cells. Other disease associations include premature chromosome condensation which is frequently observed in dividing cells from tumor tissue (Honda et al. *supra*; Murnane, J.P. (1995) *Cancer Metastasis Rev.* 14:17-29) and the significant roles of axonemal and assembly MAPs in viral pathogenesis (Sodeik, B. et al. (1997) *J. Cell Biol.* 136:1007-1021).

The discovery of new human cytoskeletal proteins and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of cell proliferative, immunological, vesicle trafficking, reproductive, smooth muscle, developmental, and nervous disorders.

SUMMARY OF THE INVENTION

The invention features substantially purified polypeptides, human cytoskeletal proteins, referred to collectively as "HCYT" and individually as "HCYT-1," "HCYT-2," "HCYT-3," "HCYT-4," "HCYT-5," "HCYT-6," "HCYT-7," and "HCYT-8." In one aspect, the invention provides a substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-8 and fragments thereof.

The invention further provides a substantially purified variant having at least 90% amino acid identity to at least one of the amino acid sequences selected from the group consisting of SEQ ID NO:1-8, and fragments thereof. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising an amino acid selected from the group consisting of SEQ ID NO:1-8 and fragments thereof. The invention also includes an isolated and purified polynucleotide variant having at least 70% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-8 and fragments thereof.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-8 and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-8 and fragments thereof.

The invention also provides a method for detecting a polynucleotide in a sample containing nucleic acids, the method comprising the steps of (a) hybridizing the complement of the polynucleotide sequence to at least one of the polynucleotides of the sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide in the sample. In one aspect, the method further comprises amplifying the polynucleotide prior to hybridization.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:9-16 and fragments thereof. The invention further provides an isolated and purified polynucleotide variant having at least 70% polynucleotide sequence identity to the polynucleotide sequence selected from the group consisting of SEQ ID NO:9-16, and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:9-16 and fragments thereof.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-8 and fragments thereof. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide, the method comprising the

steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-8 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide selected from the group consisting of SEQ ID NO:1-8 and fragments thereof. The invention also provides a purified agonist and a purified antagonist to the polypeptide.

The invention also provides a method for treating or preventing a disorder associated with decreased expression or activity of HCYT, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-8 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention also provides a method for treating or preventing a disorder associated with increased expression or activity of HCYT, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-8 and fragments thereof.

BRIEF DESCRIPTION OF THE TABLES AND FIGURES

Table 1 shows nucleotide and polypeptide sequence identification numbers (SEQ ID NO), clone identification numbers (clone ID), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding HCYT.

Table 2 shows features of each polypeptide sequence including potential motifs, homologous sequences, and methods and algorithms used for identification of HCYT.

Table 3 shows the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis, diseases or disorders associated with these tissues, and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which Incyte cDNA clones encoding HCYT were isolated.

Table 5 shows the programs, their descriptions, references, and threshold parameters used to analyze HCYT.

Figure 1 shows the amino acid sequence alignments between HCYT-8 (2195418; SEQ ID NO:8) and human p16-Arc subunit (GI 2282042; SEQ ID NO:17) produced using the multisequence alignment program of LASERGENE software (DNASTAR, Madison WI).

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"HCYT" refers to the amino acid sequences of substantially purified HCYT obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which, when bound to HCYT, increases or prolongs the duration of the effect of HCYT. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of HCYT.

An "allelic variant" is an alternative form of the gene encoding HCYT. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding HCYT include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same as HCYT or a polypeptide with at least one functional characteristic of HCYT. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of

the polynucleotide encoding HCYT, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HCYT. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HCYT. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of HCYT is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

The terms "amino acid" or "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of HCYT which are preferably at least 5 to about 15 amino acids in length, most preferably at least 14 amino acids, and which retain some biological activity or immunological activity of HCYT. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which, when bound to HCYT, decreases the amount or the duration of the effect of the biological or immunological activity of HCYT. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of HCYT.

The term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind HCYT polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize

a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

5 The term "antisense" refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and
10 the designation "positive" can refer to the sense strand.

 The term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HCYT, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

15 The terms "complementary" or "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects
20 on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

 A "composition comprising a given polynucleotide sequence" or a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or
25 amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding HCYT or fragments of HCYT may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other
30 components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

 "Consensus sequence" refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW Fragment Assembly
35 system (GCG, Madison WI). Some sequences have been both extended and assembled to produce the consensus sequence.

The term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding HCYT, by northern analysis is indicative of the presence of nucleic acids encoding HCYT in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding HCYT.

5 A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a
10 polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term "similarity" refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially
15 complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a
20 completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or
25 identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" or "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MEGALIGN program (DNASTAR, Madison WI). The
30 MEGALIGN program can create alignments between two or more sequences according to different methods, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-244.) The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of sequence A, minus
35 the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no

similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic acid sequences can also be counted or calculated by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) Methods Enzymol. 183:626-645.) Identity between sequences can also be determined by other methods known in the art, e.g., by
5 varying hybridization conditions.

“Human artificial chromosomes” (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term “humanized antibody” refers to antibody molecules in which the amino acid sequence
10 in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

“Hybridization” refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

The term “hybridization complex” refers to a complex formed between two nucleic acid
15 sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C₀t or R₀t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words “insertion” or “addition” refer to changes in an amino acid or nucleotide sequence
20 resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

“Immune response” can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of
25 various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term “microarray” refers to an arrangement of distinct polynucleotides on a substrate.

The terms “element” or “array element” in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term “modulate” refers to a change in the activity of HCYT. For example, modulation may
30 cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of HCYT.

The phrases “nucleic acid” or “nucleic acid sequence,” as used herein, refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of
35 genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this

context, "fragments" refers to those nucleic acid sequences which, comprise a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:9-16, for example, as distinct from any other sequence in the same genome. For example, a fragment of SEQ ID NO:9-16 is useful in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:9-16 from related polynucleotide sequences. A fragment of SEQ ID NO:9-16 is at least about 15-20 nucleotides in length. The precise length of the fragment of SEQ ID NO:9-16 and the region of SEQ ID NO:9-16 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment. In some cases, a fragment, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

The terms "operably associated" or "operably linked" refer to functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the sequence encoding the polypeptide but still bind to operator sequences that control expression of the polypeptide.

The term "oligonucleotide" refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. "Oligonucleotide" is substantially equivalent to the terms "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding HCYT, or fragments thereof, or HCYT itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "stringent conditions" refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent, e.g., formamide, temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

"Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of HCYT polypeptides refers to an amino acid sequence that is altered by one or more amino acid residues. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, LASERGENE software (DNASTAR).

The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to HCYT. This definition may also include, for example, "allelic" (as defined above), "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due

to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

THE INVENTION

The invention is based on the discovery of new human cytoskeletal proteins (HCYT), the polynucleotides encoding HCYT, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative, immunological, vesicle trafficking, reproductive, smooth muscle, developmental, and nervous disorders.

Table 1 lists the Incyte Clones used to derive full length nucleotide sequences encoding HCYT. Columns 1 and 2 show the sequence identification numbers (SEQ ID NO) of the amino acid and nucleic acid sequences, respectively. Column 3 shows the Clone ID of the Incyte Clone in which nucleic acids encoding each HCYT was identified, and column 4, the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones, their corresponding cDNA libraries, and shotgun sequences useful as fragments in hybridization technologies, and which are part of the consensus nucleotide sequence of each HCYT.

The columns of Table 2 show various properties of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3, potential phosphorylation sites; column 4, potential glycosylation sites; column 5, the amino acid residues comprising signature sequences and motifs; column 6, the identity of each protein; and column 7, analytical methods used to identify each protein through sequence homology and protein motifs. SEQ ID NO:10, which encodes HCYT-2, is a splice variant of SEQ ID NO:9, which encodes HCYT-1. In particular, the nucleotide sequence of SEQ ID NO:10 from nt 190 to nt 811 is identical to the nucleotide sequence of SEQ ID NO:9 from nt 319 to nt 940. The N-terminus of HCYT-1 has two leucine zipper patterns and five additional potential phosphorylation sites. HCYT-1, HCYT-2, HCYT-3, HCYT-4, HCYT-5, and HCYT-6 have chemical and structural similarity with tropomyosin isoforms. The N-termini of HCYT-5 (SEQ ID NO:5) and HCYT-6 (SEQ ID NO:6) have homology to catecholamine receptors and tektins, respectively; the N-terminus of HCYT-4 (SEQ ID NO:4) and the intervening regions of HCYT-5 and HCYT-6 have homology to tropomyosin isoforms; the C-terminus of HCYT-4 has homology to proteins which bind nucleotide di- or triphosphate molecules; and the C-termini of HCYT-5 and HCYT-6 have homology to receptors. SEQ ID NO:7 has various properties that are related to intermediate filament proteins including numerous potential phosphorylation sites, several

leucine zipper motifs, and a tektin signature sequence.

The columns of Table 3 show the tissue-specificity and disease-association of nucleotide sequences encoding HCYT. The first column of Table 3 lists the polynucleotide sequence identifiers. The second column lists tissue categories which express HCYT as a fraction of total tissues expressing HCYT. The third column lists the diseases, disorders, or conditions associated with those tissues expressing HCYT as a fraction of total tissues expressing HCYT. The fourth column lists the vectors used to subclone the cDNA library.

Figure 1 shows that chemical and structural homology, in the context of sequences and motifs, exists between HCYT-8 (SEQ ID NO:8) and human p16-Arc (GI 2282042: SEQ ID NO:17). In particular, the two proteins share 66% identity, the potential phosphorylation sites at S7 and T148, and the potential glycosylation site at N122 in HCYT-8.

The following represent selected fragments of the nucleotide sequences encoding HCYT which are useful as hybridization probes: the fragment of SEQ ID NO:15 from about nucleotide 7052 to about nucleotide 7111; and the fragment of SEQ ID NO:16 from about nucleotide 182 to about nucleotide 235.

The invention also encompasses HCYT variants. A preferred HCYT variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the HCYT amino acid sequence, and which contains at least one functional or structural characteristic of HCYT.

The invention also encompasses polynucleotides which encode HCYT. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:9-16, which encodes HCYT.

The invention also encompasses a variant of a polynucleotide sequence encoding HCYT. In particular, such a variant polynucleotide sequence will have at least about 70%, more preferably at least about 85%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HCYT. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:9-16 which has at least about 70%, more preferably at least about 85%, and most preferably at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9-16. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of HCYT.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HCYT, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance

with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HCYT, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HCYT and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring HCYT under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HCYT or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HCYT and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HCYT and HCYT derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HCYT or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:9-16 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50 % formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in

the art.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the Robbins HYDRA microdispenser (Robbins Scientific, Sunnyvale CA), Hamilton MICROLAB 2200 (Hamilton, Reno NV), Peltier Thermal Cycler 200 (PTC200; MJ Research, Watertown MA) and the ABI CATALYST 800 (Perkin-Elmer). Sequencing is then carried out using either ABI 373 or 377 DNA Sequencing Systems (Perkin-Elmer) or the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA). The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding HCYT may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and

yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-306). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HCYT may be cloned in recombinant DNA molecules that direct expression of HCYT, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HCYT.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HCYT-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation

patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding HCYT may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232.) Alternatively, HCYT itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of HCYT, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

In order to express a biologically active HCYT, the nucleotide sequences encoding HCYT or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding HCYT. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HCYT. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding HCYT and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HCYT and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences

encoding HCYT. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HCYT. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HCYT can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or pSPORT1 plasmid (Life Technologies). Ligation of sequences encoding HCYT into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HCYT are needed, e.g. for the production of antibodies, vectors which direct high level expression of HCYT may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of HCYT. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Grant et al. (1987) Methods Enzymol. 153:516-54; and Scorer, C. A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of HCYT. Transcription of sequences encoding HCYT may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HCYT may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader

sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HCYT in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of HCYT in cell lines is preferred. For example, sequences encoding HCYT can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* or *ap^r* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), β glucuronidase and its substrate β -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HCYT is inserted within a marker gene sequence, transformed cells containing sequences encoding HCYT can be identified by the absence of marker gene function. Alternatively, a

marker gene can be placed in tandem with a sequence encoding HCYT under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding HCYT and that express HCYT may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of HCYT using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HCYT is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul MN, Sect. IV; Coligan, J. E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HCYT include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HCYT, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HCYT may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HCYT may be designed to contain signal sequences which direct secretion of HCYT through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted

sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Bethesda MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HCYT may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HCYT protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HCYT activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HCYT encoding sequence and the heterologous protein sequence, so that HCYT may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, *supra*, ch 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HCYT may be achieved *in vitro* using the TNT rabbit reticulocyte lysate or wheat germ extract systems (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, preferably ³⁵S-methionine.

Fragments of HCYT may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, *supra* pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Various fragments of HCYT may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between HCYT and human cytoskeletal proteins. In addition, HCYT is expressed in tissues associated with cancer, cell proliferation, fetal development, and inflammation and the immune response, as well as in reproductive, nervous, cardiovascular, developmental, and gastrointestinal tissues. Therefore, HCYT appears to be involved with cell proliferative, immunological, vesicle trafficking, reproductive, smooth muscle, developmental, and nervous disorders. In disorders associated with decreased expression or activity of HCYT, it is desirable to increase the expression or activity of HCYT. In disorders associated with increased expression or activity of HCYT, it is desirable to decrease the expression or activity of HCYT.

Therefore, in one embodiment, HCYT or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder in which the expression or activity of HCYT is decreased. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia; cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an immunological disorder such as actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a vesicle trafficking disorder such as cystic fibrosis, glucose-galactose malabsorption syndrome, hypercholesterolemia, diabetes mellitus, diabetes insipidus, hyper- and hypoglycemia, Grave's disease, goiter, Cushing's disease, and Addison's disease; gastrointestinal disorders including ulcerative colitis, gastric and duodenal ulcers; other conditions associated with abnormal vesicle trafficking, including acquired immunodeficiency syndrome (AIDS);

allergies including hay fever, asthma, and urticaria (hives); autoimmune hemolytic anemia; proliferative glomerulonephritis; inflammatory bowel disease; multiple sclerosis; myasthenia gravis; rheumatoid and osteoarthritis; scleroderma; Chediak-Higashi and Sjogren's syndromes; systemic lupus erythematosus; toxic shock syndrome; traumatic tissue damage; and viral, bacterial, fungal, helminthic, and protozoal infections; a reproductive disorder such as disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, carcinoma of the male breast, and gynecomastia; a smooth muscle disorder such as any impairment or alteration in the normal action of smooth muscle including, but not limited to, that of the blood vessels, gastrointestinal tract, heart, and uterus, and including but not limited to, angina, anaphylactic shock, arrhythmias, asthma, cardiovascular shock, Cushing's syndrome, hypertension, hypoglycemia, myocardial infarction, migraine, and pheochromocytoma, and myopathies including cardiomyopathy, encephalopathy, epilepsy, Kearns-Sayre syndrome, lactic acidosis, myoclonic disorder, and ophthalmoplegia; a developmental disorder, such as any disorder associated with development or function of a tissue, organ, or system (such as the brain, adrenal gland, kidney, skeletal or reproductive system) of a subject, including but not limited to, renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital glaucoma, cataract, and sensorineural hearing loss; a nervous disorder such as akathisia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis and other motor neuron disorders, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, diabetic neuropathy, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, peripheral neuropathy, multiple sclerosis, neurofibromatosis, Parkinson's disease and other extrapyramidal disorders, postherpetic neuralgia, epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Pick's disease, Huntington's disease, dementia, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal

hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis; 5 inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, anxiety, paranoid psychoses, and schizophrenic disorders; diabetic neuropathy, tardive dyskinesia, dystonias, and Tourette's disorder.

In another embodiment, a vector capable of expressing HCYT or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or 10 activity of HCYT including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified HCYT in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HCYT including, but not limited to, those provided above.

15 In still another embodiment, an agonist which modulates the activity of HCYT may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HCYT including, but not limited to, those listed above.

In a further embodiment, an antagonist of HCYT may be administered to a subject to treat or prevent a disorder in which the expression or activity of HCYT is increased. Examples of such disorders 20 include, but are not limited to, those cell proliferative, immunological, vesicle trafficking, reproductive, smooth muscle, developmental, and nervous disorders listed above. In one aspect, an antibody which specifically binds HCYT may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express HCYT.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding 25 HCYT may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HCYT including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by 30 one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HCYT may be produced using methods which are generally known in the art. 35 In particular, purified HCYT may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HCYT. Antibodies to HCYT may also be generated using

methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

5 For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HCYT or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, 10 peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HCYT have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to 15 a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HCYT amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to HCYT may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited 20 to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the 25 splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HCYT-specific single chain antibodies. 30 Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the 35 literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for HCYT may also be generated. For example, such fragments include, but are not limited to, F(ab')₂ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy
5 identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such
10 immunoassays typically involve the measurement of complex formation between HCYT and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HCYT epitopes is preferred, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques
15 may be used to assess the affinity of antibodies for HCYT. Affinity is expressed as an association constant, K_a , which is defined as the molar concentration of HCYT-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple HCYT epitopes, represents the average affinity, or avidity, of the antibodies for HCYT. The K_a determined for a
20 preparation of monoclonal antibodies, which are monospecific for a particular HCYT epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the HCYT-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation
25 of HCYT, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J. E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a
30 polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is preferred for use in procedures requiring precipitation of HCYT-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

35 In another embodiment of the invention, the polynucleotides encoding HCYT, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the

polynucleotide encoding HCYT may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HCYT. Thus, complementary molecules or fragments may be used to modulate HCYT activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding HCYT.

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Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding HCYT. (See, e.g., Sambrook, *supra*; Ausubel, 1995, *supra*.)

Genes encoding HCYT can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding HCYT. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding HCYT. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HCYT.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding HCYT. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nature Biotechnology 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical or

capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

5 Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran.

10 Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

15 For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

 The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

20 The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

25 After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of HCYT, such labeling would include amount, frequency, and method of administration.

30 Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

 For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for
35 administration in humans.

 A therapeutically effective dose refers to that amount of active ingredient, for example HCYT or

fragments thereof, antibodies of HCYT, and agonists, antagonists or inhibitors of HCYT, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of therapeutic to toxic effects is the therapeutic index, and it can be expressed as the ED_{50}/LD_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μ g to 100,000 μ g, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind HCYT may be used for the diagnosis of cell proliferative, immunological, vesicle trafficking, reproductive, smooth muscle, developmental, and nervous disorders characterized by expression of HCYT, or in assays to monitor patients being treated with HCYT or agonists, antagonists, or inhibitors of HCYT. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for HCYT include methods which utilize the antibody and a label to detect HCYT in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring HCYT, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of HCYT expression. Normal or standard values for HCYT expression are established by combining body fluids or cell extracts taken

from normal mammalian subjects, preferably human, with antibody to HCYT under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of HCYT expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between
 5 standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HCYT may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of HCYT may be correlated with
 10 disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HCYT, and to monitor regulation of HCYT levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HCYT or closely related molecules may be used to identify nucleic acid sequences which encode HCYT. The specificity of the probe, whether it is made
 15 from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding HCYT, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably have at
 20 least 50% sequence identity to any of the HCYT encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of HCYT or from genomic sequences including promoters, enhancers, and introns of the HCYT gene.

Means for producing specific hybridization probes for DNAs encoding HCYT include the cloning of polynucleotide sequences encoding HCYT or HCYT derivatives into vectors for the production of
 25 mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HCYT may be used for the diagnosis of cell proliferative, immunological, vesicle trafficking, reproductive, smooth muscle, developmental, and nervous disorders associated with expression of HCYT. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria,
 35 polycythemia vera, psoriasis, primary thrombocythemia; cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal

gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an immunological disorder such as actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis fetal, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a vesicle trafficking disorder such as cystic fibrosis, glucose-galactose malabsorption syndrome, hypercholesterolemia, diabetes mellitus, diabetes insipidus, hyper- and hypoglycemia, Grave's disease, goiter, Cushing's disease, and Addison's disease; gastrointestinal disorders including ulcerative colitis, gastric and duodenal ulcers; other conditions associated with abnormal vesicle trafficking, including acquired immunodeficiency syndrome (AIDS); allergies including hay fever, asthma, and urticaria (hives); autoimmune hemolytic anemia; proliferative glomerulonephritis; inflammatory bowel disease; multiple sclerosis; myasthenia gravis; rheumatoid and osteoarthritis; scleroderma; Chediak-Higashi and Sjogren's syndromes; systemic lupus erythematosus; toxic shock syndrome; traumatic tissue damage; and viral, bacterial, fungal, helminthic, and protozoal infections; a reproductive disorder such as disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, carcinoma of the male breast, and gynecomastia; a smooth muscle disorder such as any impairment or alteration in the normal action of smooth muscle including, but not limited to, that of the blood vessels, gastrointestinal tract, heart, and uterus, and including but not limited to, angina, anaphylactic shock, arrhythmias, asthma, cardiovascular shock, Cushing's syndrome, hypertension, hypoglycemia, myocardial infarction, migraine, and pheochromocytoma, and myopathies including cardiomyopathy, encephalopathy, epilepsy, Kearns-Sayre

syndrome, lactic acidosis, myoclonic disorder, and ophthalmoplegia; a developmental disorder, such as any disorder associated with development or function of a tissue, organ, or system (such as the brain, adrenal gland, kidney, skeletal or reproductive system) of a subject, including but not limited to, renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, 5 genitourinary abnormalities, and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital 10 glaucoma, cataract, and sensorineural hearing loss; a nervous disorder such as akathisia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis and other motor neuron disorders, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, diabetic neuropathy, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, peripheral neuropathy, multiple sclerosis, neurofibromatosis, Parkinson's disease and other extrapyramidal disorders, postherpetic neuralgia, 15 epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Pick's disease, Huntington's disease, dementia, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann- 20 Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral 25 nervous system disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, anxiety, paranoid psychoses, and schizophrenic disorders; diabetic neuropathy, tardive dyskinesia, dystonias, and Tourette's disorder. The polynucleotide sequences encoding HCYT may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; 30 and in microarrays utilizing fluids or tissues from patients to detect altered HCYT expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HCYT may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding HCYT may be labeled by standard methods and added to a fluid or tissue sample 35 from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value.

If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding HCYT in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an

5 individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of HCYT, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HCYT, under conditions suitable for hybridization or amplification. Standard

10 hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

15 Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

20 With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

25 Additional diagnostic uses for oligonucleotides designed from the sequences encoding HCYT may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding HCYT, or a fragment of a polynucleotide complementary to the polynucleotide encoding HCYT, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may

30 also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of HCYT include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 229-236.) The speed of quantitation of multiple samples may be accelerated by running

35 the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a

spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding HCYT may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding HCYT on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature

336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, HCYT, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HCYT and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with HCYT, or fragments thereof, and washed. Bound HCYT is then detected by methods well known in the art. Purified HCYT can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HCYT specifically compete with a test compound for binding HCYT. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HCYT.

In additional embodiments, the nucleotide sequences which encode HCYT may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. [Atty Docket No. PF-0566 P, filed August 4, 1998], U.S. Ser. No. [Atty Docket No. PF-0568 P, filed July 31, 1998] and U.S. Ser. No.: [Atty Docket No. PF-0578 P, filed August 19, 1998], are hereby expressly incorporated by reference.

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in

phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

5 Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A⁺) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Valencia CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin
10 TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units
15 5.1-6.6). Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into
20 compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), pSPORT1 plasmid (Life Technologies), or pINCY (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 α , DH10B, or ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

25 Plasmids were recovered from host cells by in vivo excision, using the UNIZAP vector system (Stratagene) or cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the REAL Prep 96 plasmid kit from QIAGEN. Following
30 precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and
35 the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN reagent (Molecular Probes, Eugene OR) and a Fluoroskan II fluorescence scanner (Labsystems Oy, Helsinki,

Finland).

III. Sequencing and Analysis

The cDNAs were prepared for sequencing using the ABI CATALYST 800 (Perkin-Elmer) or the HYDRA microdispenser (Robbins Scientific) or MICROLAB 2200 (Hamilton) systems in combination with the PTC-200 thermal cyclers (MJ Research). The cDNAs were sequenced using the ABI PRISM 373 or 377 sequencing systems (Perkin-Elmer) and standard ABI protocols, base calling software, and kits. In one alternative, cDNAs were sequenced using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics). In another alternative, the cDNAs were amplified and sequenced using the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer). In yet another alternative, cDNAs were sequenced using solutions and dyes from Amersham Pharmacia Biotech. Reading frames for the ESTs were determined using standard methods as reviewed in Ausubel (1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example V.

The polynucleotide sequences derived from cDNA, extension, and shotgun sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the software programs, descriptions, references, and threshold parameters used. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides a brief description thereof, the third column presents the references which are incorporated by reference herein, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the probability the greater the homology). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, S. San Francisco CA) and LASERGENE software (DNASTAR).

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programing, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS to acquire annotation, using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, PFAM, and Prosite.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ database (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analyses are reported a percentage distribution of libraries in which the transcript encoding HCYT occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease categories included cancer, inflammation/trauma, fetal, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease expression are reported in Table 3.

V. Extension of HCYT Encoding Polynucleotides

Full length nucleic acid sequences (SEQ ID NO:9-14) were produced by extension of the component fragments described in Table 1, Column 5, using oligonucleotide primers based on those fragments. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO 4.06 (National Biosciences, Inc.), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

High fidelity amplification was obtained by following the instructions for the XL-PCR kit (The

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Perkin-Elmer Corp.) and thoroughly mixing the enzyme and reaction mix. PCR was performed using the PTC-200 thermal cycler (MJ Research, Inc.), beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, with the following parameters:

5	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
	Step 3	68° C for 6 min
	Step 4	94° C for 15 sec
	Step 5	65° C for 1 min
	Step 6	68° C for 7 min
10	Step 7	Repeat steps 4 through 6 for an additional 15 cycles
	Step 8	94° C for 15 sec
	Step 9	65° C for 1 min
	Step 10	68° C for 7:15 min
	Step 11	Repeat steps 8 through 10 for an additional 12 cycles
15	Step 12	72° C for 8 min
	Step 13	4° C (and holding)

A 5 μ l to 10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using QIAQUICK™ (QIAGEN Inc.), and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13 μ l of ligation buffer, 1 μ l T4-DNA ligase (15 units) and 1 μ l T4 polynucleotide kinase were added, and the mixture was incubated at room temperature for 2 to 3 hours, or overnight at 16° C. Competent *E. coli* cells (in 40 μ l of appropriate media) were transformed with 3 μ l of ligation mixture and cultured in 80 μ l of SOC medium. (See, e.g., Sambrook, *supra*, Appendix A, p. 2.) After incubation for one hour at 37°C, the *E. coli* mixture was plated on Luria Bertani (LB) agar (See, e.g., Sambrook, *supra*, Appendix A, p. 1) containing carbenicillin (2x carb). The following day, several colonies were randomly picked from each plate and cultured in 150 μ l of liquid LB/2x carb medium placed in an individual well of an appropriate commercially-available sterile 96-well microtiter plate. The following day, 5 μ l of each overnight culture was transferred into a non-sterile 96-well plate and, after dilution 1:10 with water, 5 μ l from each sample was transferred into a PCR array.

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

40	Step 1	94° C for 60 sec
	Step 2	94° C for 20 sec
	Step 3	55° C for 30 sec
	Step 4	72° C for 90 sec
	Step 5	Repeat steps 2 through 4 for an additional 29 cycles
	Step 6	72° C for 180 sec
	Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

5 In like manner, the nucleotide sequence of SEQ ID NO:9-14 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

The full length nucleic acid sequences of SEQ ID NO:15-16 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other
10 primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

15 Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+} , $(NH_4)_2SO_4$, and β -
20 mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step
25 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 μ l PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes) dissolved in 1X TE and 0.5 μ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA),
30 allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 μ l to 10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates,
35 digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun

sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and
5 transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated
10 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulphoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit
15 (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:15-16 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

20 VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:9-16 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National
25 Biosciences, Inc.) and labeled by combining 50 pmol of each oligomer, 250 μ Ci of [γ -³²P] adenosine triphosphate (Amersham Pharmacia Biotech, Ltd.), and T4 polynucleotide kinase (DuPont NEN, Boston, MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech, Ltd.). An aliquot containing 10⁶ counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human
30 genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN, Boston, MA).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (NYTRAN Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under
35 increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR film (Eastman Kodak, Rochester, NY) is exposed to the blots, hybridization patterns are

compared visually.

VII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

VIII. Complementary Polynucleotides

Sequences complementary to the HCYT-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HCYT. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of HCYT. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HCYT-encoding transcript.

IX. Expression of HCYT

Expression and purification of HCYT is achieved using bacterial or virus-based expression systems. For expression of HCYT in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express HCYT upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of

HCYT in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding HCYT by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates.

5 Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

10 In most expression systems, HCYT is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech).

15 Following purification, the GST moiety can be proteolytically cleaved from HCYT at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch 10 and 16).

20 Purified HCYT obtained by these methods can be used directly in the following activity assay.

X. Demonstration of HCYT Activity

HCYT activity may be measured by effects of the proteins on cellular locomotion. In vitro cell motility (locomotion) assays are performed as follows. Myosin is diluted to 200 µg/ml in buffer C (25 mM imidazole, pH 7.4, 25 mM KCl, 4 mM MgCl₂, 1 mM EGTA, 10 mM dithiothreitol), applied to a flow
25 cell coated with nitrocellulose, and blocked with buffer C containing 0.5 mg/ml BSA (C/BSA). A solution of phalloidin-labeled actin is perfused followed by 1 mM ATP in C/BSA to remove myosin heads that bind actin in a rigor fashion. After washing with C/BSA to remove the excess nonfluorescent actin, a solution of rhodamine-phalloidin-labeled actin and HCYT in C/BSA is introduced. Active movement is initiated at room temperature by introducing C/BSA containing 1 mM ATP and oxygen
30 scavenger enzymes. Images (recorded using a Zeiss standard microscope (Zeiss, New York NY) equipped with a Hamamatsu SIT camera) of moving myotubes are tracked for up to 30 sec, and translocation velocities calculated using the myotube centroids to establish initial and final positions for 2 sec or 4 sec samples during the continuous movement.

Alternatively, an assay for HCYT activity measures the binding affinity of HCYT for actin as
35 described by Hammell, R.L. and Hitchcock-DeGregori, S.E. (1997, J. Biol. Chem. 272:22409-22416). HCYT and actin are prepared from in vitro recombinant cDNA expression systems and the N-terminus of

HCYT is acetylated using methods well known in the art. Binding of N-terminal acetyl-HCYT to actin is measured by cosedimentation at 25°C in a Beckman model TL-100 centrifuge as described. The bound and free HCYT are determined by quantitative densitometry of SDS-polyacrylamide gels stained with Coomassie Blue. Apparent binding constants (K_{app}) and Hill coefficients (H) are determined by using methods well known in the art to fit the data to the equation as described by Hammell and Hitchcock-DeGregori (1997, supra). The HCYT:actin ratio determined using densitometry is normalized. Hammell and Hitchcock-DeGregori (1997, supra) have shown that saturation of binding corresponds to a HCYT:actin molar ratio of 0.14, a stoichiometry of 1 HCYT:7 actin.

10 **XI. Functional Assays**

HCYT function is assessed by expressing the sequences encoding HCYT at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10 μ g of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2 μ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP, and to evaluate properties, for example, their apoptotic state. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of HCYT on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding HCYT and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA

encoding HCYT and other genes of interest can be analyzed by northern analysis or microarray techniques.

XII. Production of HCYT Specific Antibodies

HCYT substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the HCYT amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, *supra*, ch. 11.)

Typically, oligopeptides 15 residues in length are synthesized using an ABI 431A Peptide Synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, *supra*.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity by, for example, binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIII. Purification of Naturally Occurring HCYT Using Specific Antibodies

Naturally occurring or recombinant HCYT is substantially purified by immunoaffinity chromatography using antibodies specific for HCYT. An immunoaffinity column is constructed by covalently coupling anti-HCYT antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HCYT are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HCYT (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HCYT binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HCYT is collected.

XIV. Identification of Molecules Which Interact with HCYT

HCYT, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) *Biochem. J.* 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HCYT, washed, and any wells with labeled HCYT complex are assayed. Data obtained using different concentrations of HCYT are used to calculate values for the number, affinity, and association of HCYT with the candidate molecules.

Various modifications and variations of the described methods and systems of the invention will

be apparent to those skilled in the art without departing from the scope and spirit of the invention.

Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

Indeed, various modifications of the described modes for carrying out the invention which are obvious to
5 those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Protein SEQ ID NO:	Nucleo- tide SEQ ID NO:	Clone ID	Library	Fragments
1	9	1274060	TESTTUT02	1900209T6 (BLADTUT06), 1909056F6 (CONNTUT01), SAEA01408F1, SAEA02983R1
2	10	1577078	LNODNOT03	1577078H1 (LNODNOT03), 684408R6 (UTRSNOT02), 2478839T6 (SMCANOT03), 1909056F6 (CONNTUT01)
3	11	1426711	SINTBST01	1426711H1 (SINTBST01), 3749812H1 (UTRSNOT18), 3094943H1 (CERVNOT03), 2859695H1 (SININOT03), 4705751H1 (SMCRTXT01)
4	12	1676756	STOMFET01	760671R1 (BRAITUT02), 1676756H1 and 1676756T6 (STOMFET01), 2080585F6 (UTRSNOT08), 2364126F6, 2364126H1, and 2364126T6 (ADRENOT07), 2735685H1 (OVARNOT09), 2811007H1 (OVARNOT10)
5	13	1843770	COLNNOT08	262277R6 and 262277T6 (HNT2AGT01), 1509335F1 (LUNGNOT14), 1620333F6 (BRAITUT13), 1843770H1, 1843770X11, 1843770X13, 1843770X14, and 1843770X21 (COLNNOT08)
6	14	3768043	BRSTNOT24	288738H1 (EOSIHET02), 1328455F1 (PANCNOT07), 1340936F1 and 1340936T1 (COLNTUT03), 1695275F6 (COLNNOT23), 2106351R6 (BRAITUT03), 2378482F6 and 2378482T6 (ISLTNOT01), 3768043H1 (BRSTNOT24), 3988936H1 (LUNGNON03)
7	15	1655208	PROSTUT08	749902H1 (BRAITUT01), 755160H1 (BRAITUT02), 1457865H1 (COLNFET02), 1486091H1 (CORPNOT02), 1574402T6 (LNODNOT03), 1595969H1 (BRAINOT14), 1655208H1 (PROSTUT08), 1705982F6, 1705982H1, 1706039F6, and 1706039T6 (DUODNOT02), 1732192F6 (BRSTTUT08), 1850069H1 (LUNGFET03), 2363256X303D1 (LUNGFET05), 3036180H1 (BONEUNT01), 3093057H1 (BRSTNOT19), 4737159H1 (THYMNOR02)
8	16	2195418	THP1NOT01	024620R1 (ADENINB01), 1454159H1 (PENITUT01), 1797859R6 (PROSTUT05), 1943527T6 (HIPONOT01), 2195418H1 (THP1NOT01)

Table 2

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence	Identification	Analytical Methods
1	284	S79 T53 S215 T237 T252 T277 T74 S123 S211 S229	N199	L43-L64 L50-L71 L232-E240	tropomyosin	Blast BLOCKS PRINTS PROFILESCAN Pfam Motifs
2	158	S89 T111 T126 T151 S85 S103	N73	L106-E114	tropomyosin	Blast BLOCKS PRINTS PROFILESCAN Pfam Motifs
3	208	S53 S174 S123			tropomyosin	Blast BLOCKS PRINTS PROFILESCAN Pfam
4	156	S82 T86 T13 S36 S64		K38 to N52 E54 to L102	tropomyosin	BLOCKS PRINTS
5	876	S385 T25 T106 T168 T236 S271 T273 S329 T345 S387 S463 S479 T593 T621 S654 T665 S711 S859 T172 S324 T560 S682 T778 T808 S848 Y735 Y835	N104 N343	E199 to V247 E223 to Q260	tropomyosin, receptors, liprin (coiled-coil LAR- interacting protein)	Blast BLOCKS PRINTS Motifs

Table 2, cont'd.

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence	Identification	Analytical Methods
6	806	T403 S110 S111 S138 S140 S161 S176 T247 T287 T288 S299 S343 T348 T371 S417 T491 T541 S579 S615 S659 S664 T668 S29 S62 S65 S183 T197 S222 S327 T432 S633 S663 Y37 Y408	N136 N325 N666	E133 to S181 E413 to S450	tropomyosin, receptors, meningioma expressed antigen 6	Blast BLOCKS PRINTS Motifs
7	2442	S157 S697 S112 S196 T198 S272 S363 S377 S538 T544 S576 S581 S631 T662 T708 S764 S772 S781 S820 T842 T896 T910 T923 S929 T937 S1060 S1161 S1195 S1199 T1274 T1276 S1302 S1397 T1429 S1487 S1634 S1691 S1774 S1803 T1853 S2095 S2132 S2189 S2200 S2234 S2240 S2252 S2298 T2334 T2380 T2405 S38 T186 S235 S295 T396 T586 T817 S1025 S1076 T1383 T1439 T1519 S1540 T1585 T1701 S1951 S2128 S2138 T2218 S2267 T2411 Y1043	N233 N319 N626 N1352	Leucine Zipper: L309 to L330 L433 to L496 L580 to L622 L994 to L1015 L2275 to L2296 Tektin Signature: E1747 to H1763	centrosomal protein	Blast PRINTS Motifs
8	153	S42 S91 S7 T148	N122		p16-Arc	Blast

09744314-070201

Table 3

SEQ ID NO:	Tissue Expression (Fraction of Total)	Disease Class (Fraction of Total)	Vector
9	Hematopoietic/Immune (0.216) Cardiovascular (0.189) Reproductive (0.162)	Cancer (0.378) Inflammation (0.378) Fetal (0.216)	pINCY
10	Hematopoietic/Immune (0.250) Cardiovascular (0.188) Developmental (0.125)	Inflammation (0.406) Cancer (0.375) Fetal (0.219)	pINCY
11	Reproductive (0.371) Gastrointestinal (0.236) Cardiovascular (0.157)	Cancer (0.521) Inflammation (0.164) Trauma (0.136)	pINCY
12	Nervous (0.267) Hematopoietic/Immune (0.150) Cardiovascular (0.133) Reproductive (0.133)	Cancer (0.500) Inflammation (0.366) Fetal (0.117)	pINCY
13	Nervous (0.233) Gastrointestinal (0.150) Reproductive (0.167)	Cancer (0.600) Inflammation (0.283) Fetal (0.133)	PSPORT1
14	Reproductive (0.283) Gastrointestinal (0.185) Hematopoietic/Immune (0.120)	Cancer (0.551) Inflammation (0.357) Fetal (0.212)	pINCY
15	Reproductive (0.243) Nervous (0.162) Developmental (0.135)	Cancer (0.514) Fetal (0.270) Inflammation (0.216)	pINCY
16	Nervous (0.270) Reproductive (0.254) Hematopoietic/Immune (0.159)	Cancer (0.365) Inflammation (0.365) Fetal (0.222)	pINCY

Table 4

Nucleo- tide SEQ ID NO:	Clone ID	Library	Library Comment
9	1274060	TESTTUT02	Library was constructed using RNA isolated from testicular tumor removed from a 31-year-old Caucasian male during unilateral orchiectomy. Pathology indicated embryonal carcinoma.
10	1577078	LNODNOT03	Library was constructed using RNA isolated from lymph node tissue obtained from a 67-year-old Caucasian male during a segmental lung resection and bronchoscopy. On microscopic exam, this tissue was found to be extensively necrotic with 10% viable tumor. Pathology for the associated tumor tissue indicated invasive grade 3-4 squamous cell carcinoma. Patient history included hemangioma. Family history included atherosclerotic coronary artery disease, benign hypertension, congestive heart failure, atherosclerotic coronary artery disease.
11	1426711	SINTBST01	Library was constructed using RNA isolated from ileum tissue obtained from an 18-year-old Caucasian female during bowel anastomosis. Pathology indicated Crohn's disease of the ileum, involving 15 cm of the small bowel. Family history included cerebrovascular disease and atherosclerotic coronary artery disease.
12	1676756	STOMFET01	Library was constructed using RNA isolated from the stomach tissue of a Caucasian female fetus, who died at 20 weeks gestation.
13	1843770	COLNNOT08	Library was constructed using RNA isolated from colon tissue removed from a 60-year-old Caucasian male during a left hemicolectomy.
14	3768043	BRSTNOT24	Library was constructed using RNA isolated from diseased breast tissue removed from a 46-year-old Caucasian female during bilateral subcutaneous mastectomy. Pathology indicated nonproliferative fibrocystic disease. Family history included breast cancer and cardiovascular disease.

Table 4, cont'd.

Nucleo- tide SEQ ID NO:	Clone ID	Library	Library Comment
15	1655208	PROSTUT08	Library was constructed using RNA isolated from prostate tumor tissue removed from a 60-year-old Caucasian male during radical prostatectomy and regional lymph node excision. Pathology indicated an adenocarcinoma (Gleason grade 3+4). Adenofibromatous hyperplasia was also present. The patient presented with elevated prostate specific antigen (PSA). Family history included tuberculosis, cerebrovascular disease, and arteriosclerotic coronary artery disease.
16	2195418	THPINOT01	Library was constructed using RNA isolated from untreated THP-1 cells. THP-1 (ATCC TIB 202) is a human promonocyte line derived from the peripheral blood of a 1-year-old Caucasian male with acute monocytic leukemia (Int. J. Cancer (1980) 26:171).

Table 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS and PRINTS databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff, Nucl. Acid Res., 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and Probability value= 1.0E-3 or less
PFAM	A Hidden Markov Models-based application useful for protein family search.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits, depending on individual protein families

Table 5 cont.

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Score= 4.0 or greater
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Clavette, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. <i>supra</i> ; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	